IN THE SPECIFICATION:

Please replace paragraph **[0008]** of the as-filed specification with the following amended paragraph:

Human lactoferrin has been used as a marker for fecal leukocytes in a number of applications. For instance, fecal lactoferrin has been used as a marker for leukocytes to distinguish noninflammatory diarrhea from inflammatory diarrhea, as disclosed in U.S. Patent No. 5,124,252 (the "'252 patent"). Noninflammatory diarrhea caused by agents such as rotavirus, Norwalk-like agents and cholera, typically causes minimal to no intestinal damage and patients respond readily to oral rehydration. Inflammatory diarrheas include those caused by enteric pathogens such as Clostridium difficile, Shigella species, Salmonella species, Campylobacter jejuni and Entamoeba histolytica and those that have no clearly defined infectious agent such as CD and UC. U.S. Patent No. 5,124,252 discloses an in vitro test for fecal leukocytes which aids in distinguishing inflammatory from noninflammatory diarrhea. The ~~>252~~'252 patent discloses testing fecal samples suspected of containing leukocytes with an assay that utilizes an antibody for lactoferrin to determine the presence of leukocytes in the fecal sample.

Please replace paragraph **[0009]** of the as-filed specification with the following amended paragraph:

Human lactoferrin also has been used as a marker for diagnosis of inflammatory gastrointestinal disorders, colon polyp and colorectal cancer as disclosed in U.S. Patent No. 5,552,292 (the "'292 patent"). However, neither the method of the ~~>252~~'252 patent nor that of the ~~>292~~'292 patent disclose utility in distinguishing IBS and IBD. The samples tested by the

assay of the ~~'252~~'252 patent are samples suspected of containing leukocytes. This suspicion is owed to the patient presenting with diarrhea. However, 25-50% of persons having IBD do not present with diarrhea and, thus, the ~~'252~~'252 patent does not relate to diagnosing etiology in such patients. As for the ~~'292~~'292 patent, the disclosed method utilizes a 1:100 sample dilution which does not allow for accurate quantitation of lactoferrin levels. Further, the ~~'292~~'292 patent discloses using partial forms of molecules for testing and not total endogenous lactoferrin, again affecting the accuracy of the quantitation. The method of the ~~'292~~'292 patent also does not relate to utilizing lactoferrin levels to distinguish between IBD and IBS. The population tested in the ~~'292~~'292 patent, while including persons with UC and CD, did not include persons having IBS. Therefore, there remains a need in the diagnostic industry for a noninvasive method for differentially diagnosing IBD and IBS which utilizes human lactoferrin as a marker.

Please replace paragraph **[0013]** of the as-filed specification with the following amended paragraph:

Accordingly, the present invention provides a non-invasive method for differentiating irritable bowel syndrome (IBS) from inflammatory bowel disease (IBD) wherein the presence of fecal lactoferrin is used as a detection marker for fecal leukocytes, elevated levels of which substantially preclude diagnoses of IBS and other noninflammatory etiologies, and a kit therefor. This rapid diagnosis then may be utilized by healthcare professionals to prescribe proper treatment. The present invention further provides immunoassays, e.g., enzyme-linked immunoassays (ELISAs), that utilize antibodies specific to human lactoferrin for the measurement of total endogenous lactoferrin in clinical specimens, such as human feces, mucus and bile, and a kit usable in such immunoassays. Still further, the present invention provides ~~to~~ a

method for quantitating the levels of lactoferrin from endogenous sources, particularly, infiltrating leukocytes, to monitor gastrointestinal inflammation in persons having IBD.

Please replace paragraph **[0016]** of the as-filed specification with the following amended paragraph:

For the evaluation of the qualitative assay of the present invention as a diagnostic aid for IBD and IBS patients, fecal samples from subjects having IBD were collected and the assay results were compared with those from healthy control subjects and subjects having clinically defined cases of IBS. The IBD group included subjects having both ulcerative colitis (UC) and Crohn's disease (CD). The fecal lactoferrin levels determined in these subjects were used to establish the preferred predictive optical density for the assay of 0.200 OD_{450}. Results indicated that the assay was positive (i.e., an OD_{450} greater than or equal to 0.200) for 86.0% of fecal specimens from subjects with active IBD and was consistently negative (i.e., an OD_{450} less than 0.200) for specimens from subjects with active IBS and from healthy control subjects. ~~("OD$_{450}$"~~ "OD_{450}" as used herein indicates an optical density measured at 450 nm on a single wavelength spectrophotometer.)

Please replace paragraph **[0019]** of the as-filed specification with the following amended paragraph:

Also provided is a quantitative ELISA wherein polyclonal antibodies against total endogenous human lactoferrin are utilized to ~~quantitative~~quantitate levels of gastrointestinal inflammation through comparison to a standard curve generated using purified human lactoferrin. These levels then may be utilized to monitor the effects of medical treatments in patients having IBD.

Please replace paragraph **[0030]** of the as-filed specification with the following amended paragraph:

Standard collection and handling procedures typically used for fecal specimens for culture may be used in collecting samples for the assay of the present invention. In the preferred embodiment, fecal specimens are to be tested within twenty-four hours of collection. However, if the assay is not to be performed within forty-eight hours of collection, it is preferred that the specimens be stored at -20~~EC~~°C or lower. Additionally, it is preferred that collected specimens be transported and diluted in the Diluent as soon as possible after collection and, once diluted, that the specimens be stored at between about 2~~EC~~°C and about 8~~EC~~°C. It is preferred that the specimens be mixed (i.e., using a vortex mixer) thoroughly prior to performing the assay of the present invention. This includes complete mixing of the specimen prior to transfer to the Diluent, as more fully described below, as well as complete mixing of the diluted specimen prior to performing the assay.

Please replace paragraph **[0033]** of the as-filed specification with the following amended paragraph:

The specimen in the second tube prepared according to either of the above procedures was mixed in a vortex mixer for approximately ten seconds and subsequently stored at between about 2~~EC~~°C and about 8~~EC~~°C until the remainder of the test procedure was performed. Prior to transferring the diluted specimen into a microtiter well according to the test procedure, as more fully described below, the specimen was thoroughly mixed in the vortex mixer once again. This procedure sought to ensure thorough mixing of the specimen.

Please replace paragraph **[0034]** of the as-filed specification with the following amended paragraph:

A number of reagents were necessary to carry out the preferred embodiment of the qualitative assay of the present invention. These reagents included 10X Diluent, 1X Diluent, Conjugate, Substrate, Positive Control, Wash Buffer Solution and Stop Solution. The 10X Diluent was a 10X concentrate of buffered protein solution containing 0.2% thimerosal as a preservative. The Diluent was supplied as a 10X concentrate. Therefore, to prepare the 1X Diluent necessary for the assay of the present invention, a total volume of 400 mL was diluted by adding 40 mL of the 10X concentrate to 360 mL of deionized water. Any unused 1X Diluent was stored at between about 2~~EC~~°C and about 8~~EC~~°C.

Please replace paragraph **[0036]** of the as-filed specification with the following amended paragraph:

The Wash Buffer Solution used with the assay of the present invention was supplied as a 20X concentrate containing phosphate buffered saline, detergent and 0.2% thimerosal as a preservative. To prepare the 1X Wash Solution necessary for the assay of the present invention, a total volume of one liter of concentrate was diluted by adding 50 mL of the concentrate to 950 mL of deionized water. Any unused 1X Wash Solution was stored at between about 2~~EC~~°C and about 8~~EC~~°C.

Please replace paragraph **[0040]** of the as-filed specification with the following amended paragraph:

To perform the qualitative assay of the present invention, initially the number of wells needed was determined. Each specimen or control required one well and, therefore, the number

of wells was determined accordingly. Next, one drop (i.e., about 50 µL) of Positive Control was added to a single well designated the Positive Control Well and one drop (i.e., about 50 µL) of 1X Diluent was added to a single well designated the Negative Control Well. Subsequently, two drops (i.e., about 100 µL) of 1:400 diluted specimen (prepared according to the above procedure) was added to a third well and all wells were incubated at about 37~~EC~~°C (~~" 2EC~~)(±2°C) for approximately thirty minutes. After incubation, the contents of the assay wells was discarded into a discard pan.

Please replace paragraph **[0042]** of the as-filed specification with the following amended paragraph:

Subsequently, one drop (i.e., about 50µL) of Conjugate was added to each well and the wells were incubated at about 37~~EC~~°C (~~" 2EC~~)(±2°C) for approximately thirty minutes. After incubation, the contents of the assay wells were discarded into a discard pan and the washing procedure was repeated. Next, two drops (i.e., about 100 µL) of Substrate were added to each well and the wells were gently tapped to mix the contents. The wells were then incubated at room temperature for approximately fifteen minutes. The wells were gently tapped a couple of times during the incubation period.

Please replace paragraph **[0043]** of the as-filed specification with the following amended paragraph:

Next, one drop (i.e., 50 µL) of Stop Solution was added to each well and the wells were gently tapped. The wells were allowed to sit at room temperature for about two minutes before reading. The addition of Stop Solution converted the blue color to a yellow color which could then be quantified by measuring the optical density at 450 nm on a microplate ELISA reader.

The instrument was blanked against the negative control and the underside of each well was wiped before measuring the optical density. Optical densities (OD_{450} and $OD_{450/620}$) were recorded for the Positive Control Well, the Negative Control Well and each specimen tested. (~~"$OD_{450/620}$"~~"$OD_{450/620}$" as used herein indicates an optical density obtained spectrophotometrically at 450/620 nm on a dual wavelength spectrophotometer.) Readings of duplicate wells were averaged before the results were interpreted.

Please replace paragraph **[0051]** of the as-filed specification with the following amended paragraph:

Fecal specimens were collected from each enrolled subject and stored at -70~~EC~~°C until tested. Sample consistencies ranged from liquid to solid, numbers for which are illustrated in Table X for each subject group. As can be seen, forty-five of the IBD specimens were liquid specimens, sixty-two were semi-solid specimens, and forty-two were solid specimens. One of the IBS specimens was a liquid specimen, thirteen were semi-solid specimens, and seventeen were solid specimens. All of the specimens from healthy control subjects were solid.

Please replace paragraph **[0058]** of the as-filed specification with the following amended paragraph:

In the quantitative assay of the present invention, fecal specimens preferably are serially diluted ten-fold and added to microtiter wells containing immobilized polyclonal antibodies against human lactoferrin. If endogenous lactoferrin is present, it will bind to the antibodies during an incubation at approximately 37~~EC~~°C. Following the incubation, conjugate comprised of polyclonal antibodies coupled to horseradish peroxidase enzyme is added and allowed to bind to captured lactoferrin. Unbound conjugate is then washed from the well and a component

substrate (e.g., tetra-methyl-benzidene and hydrogen peroxide) is added for color development. Following the substrate incubation, 0.6N sulfuric acid is added to quench the reaction and the absorbance or optical density (OD) is obtained spectrophotometrically at 450 nm on a single wavelength device. Fecal lactoferrin concentrations are determined by comparison to a standard curve generated using purified human lactoferrin.